



UNIVERSITI PUTRA MALAYSIA

**MOLECULAR CHARACTERIZATION OF COAGULASE POSITIVE
STAPHYLOCOCCI ISOLATED FROM DOGS AND CATS**

OMER HASSAN MOHAMED HASSAN ARABI

FPV 2001 3

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By

OMER HASSAN MOHAMED HASSAN ARABI

**Thesis Submitted in Fulfilment of the Requirement of the Degree of Doctor
of Philosophy in the Faculty of Veterinary Medicine
Universiti Putra Malaysia**

September 2001



DEDICATION

*TO MY LATE PARENTS AND ALL MEMBERS OF MY FAMILY
TO MY DEAR BROTHER OTHMAN
TO MY WIFE FIROZE AND MY DAUGHTER TWASUL*

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: Abdul Rahim Abdul Mutalib, DVM, Ph.D.

Faculty: Veterinary Medicine

Isolation studies of coagulase-positive staphylococci were conducted on hospitalised and out patient dogs and cats, on three sites: skin, nose and ear, between January and September 1997. Three tests were used to differentiate between coagulase-positive staphylococci, namely: acetoin production, P agar supplemented with acriflavin and β -galactosidase test. Thirty-six *Staphylococcus aureus* and 90 *Staphylococcus intermedius* isolates were recovered from these animals. *Staphylococcus hyicus* was not isolated, the results indicated that the major coagulase-positive *Staphylococcus* species in dogs was *S. intermedius* and in cats was *S. aureus*.

The antimicrobial typing of *S. aureus* and *S. intermedius* was compared with the molecular typing methods such as: Polyacrylamide-gel Electrophoresis profiles of protein A concentration ^{and} whole cell proteins, and Polymerase Chain Reaction



(PCR)-based methods that include: Random Amplification of Polymorphic DNA (RAPD-PCR), Enterobacterial Repetitive Intergenic Consensus sequences (ERIC-PCR), Coagulase gene PCR amplification and Restriction Fragment Length Polymorphism (RFLP).

The antimicrobial typing differentiated *S. aureus* and *S. intermedius* isolates into 14 and 28 profiles respectively. Isolates of *S. aureus* and *S. intermedius* containing plasmids were 41.7% and 46% respectively. However, no correlation could be made between plasmid occurrence and antibiotic resistance profiles. The SDS-PAGE profiles of whole cell proteins grouped 24 *S. aureus* and 48 *S. intermedius* strains into 19 and 16 profiles respectively.

In PCR-based methods the isolates were typed using three primers. The combination of three primers for the RAPD gave 33 and 83 profiles of 36 *S. aureus* and 90 *S. intermedius* isolates respectively. ERIC primers grouped 24 *S. aureus* and 47 *S. intermedius* isolates into 19 and 43 profiles respectively. The coagulase gene from 24 *S. aureus* and 47 *S. intermedius* isolates showed limited discriminatory to the other methods and was least useful for the preliminary epidemiological studies. The restriction enzyme analysis of coagulase gene PCR products was very useful to increase the discriminatory power of coagulase gene PCR but required the use of multiple restriction enzymes. It was concluded that RAPD-PCR and ERIC-PCR are the best methods for typing *S. aureus* and *S. intermedius*.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENCIRIAN MOLEKUL STAFILOKOKUS POSITIF KOAGULASE
DIPENCIL DARIPADA ANJING DAN KUCING**

Oleh

OMER HASSAN MOHAMED

September 2001

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Kajian pemencilan stafilocokus-positif telah dijalankan di antara bulan Januari dan September 1997, terhadap anjing dan kucing pesakit luar dan hospital, pada tiga tapak: kulit, hidung, dan telinga. Tiga ujian telah diguna untuk membeza di antara stafilocokus koagulase-positif iaitu, penghasilan aseton, ujian agar P ditambah akriflavin dan β -galaktosidase. Tiga puluh enam pencilan *Staphylococcus aureus* dan 90 pencilan *Staphylococcus intermedius* telah diperolehi daripada haiwan tersebut. Hasil kajian menunjukkan yang spesies *Staphylococcus* utama dalam anjing ialah *S. intermedius* dan dalam kucing *S. aureus*.

Pengetipan antimikrob *S. aureus* dan *S. intermedius* telah dibandingkan melalui kaedah pengetipan molekul seperti: profil Elektroforesis Poliakrilamida-gel protein dan protein A sel sepenuh, dan kaedah berasas Tindak Balas Rangkaian Polimerase (PCR): Penguatan Rawak DNA Polimorfik (RAPD-PCR), jujukan

Konsensus Antaragen Berulang Enterobakteria (ERIC-PCR), Penguatan PCR gen Koagulase dan Polimorfisme Panjang Fragmen Pengehadan (RFLP)

Pengetipan antibiogramer dapat membezakan pencilan *S. aureus* dan *S. intermedius* yang mengandungi plasmid masing-masing 41.7% dan 46%. Bagaimanapun tiada sebarang perkaitan berlaku di antara kewuiudan plasmid dengan profil ketahanan antibiotik. Profil SDS-PAGE protein sel sepenuh telah mengumpulakan 24 strain *S. aureus* dan 48 strain *S. intermedius* masing-masing kepada 19 dan 16 profil.

Dalam kaedah berasaskan PCR, strain boleh ditipkan dengan mengguna tiga primer. Gabungan tiga primer untuk RAPD memberikan 33 dan 83 profil masing-masing daripada 36 strain *S. aureus* dan 90 strain *S. intermedius*. Primer ERIC mengumpulkan 24 strain *S. aureus* dan 47 strain *S. intermedius* masing-masing kepada 19 dan 43 profil. Gen koagulase daripada 24 strain *S. aureus* dan 47 strain *S. intermedius* menunjukkan pembezaan terhad terhadap kaedah lain dan paling kurang kegunaannya dalam kajian epidemiologi awal. Analisis enzim pengehadan hasil PCR gen koagulase adalah paling tinggi kegunaannya untuk meningkatkan kuasa pembezaan PCR gen koagulase, tetapi ia memerlukan penggunaan enzim pengehadan berbilang. Kesimpulannya ialah, RAPD-PCR dan ERIC-PCR merupakan kaedah paling baik untuk mengetip *S. aureus* dan *S. intermedius*.

ACKNOWLEDGEMENTS

All praise to Allah, the Merciful and the Benevolent. Had it not been due to His will and favour, the completion of this work would not have been possible.

I would like to express my sincere gratefulness and appreciation to my supervisor, Dr. Abdul Rahim Abdul Mutalib, who has devoted a lot of his time for invaluable guidance, advice, supervision and support throughout the course of this study.

I wish to express my sincere gratitude to the Dean of the faculty and my co-supervisor Professor Dato Dr. Sheikh Omar Abdul Rahman for his invaluable advice, support and continued encouragement towards the completion of this work and being helpful whenever I ran into difficulties.

Sincere thanks also to my co-supervisors, Associate Professor Dr. Saleha Abdul Aziz and Associate Professor Dr. Son Radu who have provided advice and helpful discussion that have enlightened and improved this study.

I would like to express my gratitude to Professor Dr. Abdul Rani Bahaman who had provided facilities of his laboratory during the course of this study.

I would like to thank the staff members of Veterinary Clinic and Hospital, Dr. Habibah Arshad, Dr. Vijayndra Madawar, Pn Halimah Abu Bakar and Pn Hasina Behgam. I wish to thank Mr Shahreer at the Laboratory animal's farm for his kind assistance. I deeply send my "Duaa" to our late helpful man Mr. Basri Kasim, I asked Allah to forgive him and ascend mercies to his grave and enlighten it for him. My deeply indebted thanks to Mr Hajariah Salamah that was very helpful and generous with his time. I am very grateful with Mr Fauzi Che Yosof for his skilled assistance during this study.

Also, I had been very fortunate in receiving assistance from a number of my colleagues and friends. They had to give up some of their valuable time to help me and it would not be possible to name all of them. However, I would like to thank Mr. Omer Hussabo, Dr. Izz Eldeen Babeker, Dr. Esam Mohamed Ali, Mr Elwaleed Awad, Mr. Eltahir Siddig, Mr Tarig Elsharif, Mr Mohamed Ghersi, Mr Naseer Eldeen Elhadi, Pn Nemita Fefadrah, Mr Samuel Lihan, Pn Zunita Zakaria, Pn Siti Khirani and Ooi Wai Ling.

Lastly, I would like to thank my brother Othman Arabi for his unlimited support to me during my study. Grateful thanks to my supportive wife, Firoze Mustafa Suliman and my daughter Twasul who had to put up with my long absence from them during study, so that I could complete this work.

I certify that an Examination Committee met on 7th September 2001 to conduct the final examination of Omer Hassan Mohamed Hassan Arabi on his Doctor of Philosophy thesis entitled "Molecular Characterization of Coagulase Positive Staphylococci Isolated from Dogs and Cats" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members for the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously submitted for any other degree at UPM or other institutions.



Omer Hassan Mohamed Hassan Arabi

Date: 7-11-2001

TABLE OF CONTENTS

DEDICATION	Page ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL SHEETS	ix
DECLARATION FORM	xi
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xxviii

Chapter

1	INTRODUCTION	1
2	LITERATURE REVIEW	6
	2.1 Introduction	6
	2.2 Taxonomy	7
	2.3 Coagulase-Positive Staphylococcal Virulence	8
	2.3.1 Exoenzymes	9
	2.3.2 Toxins	11
	2.4 Infections Caused by <i>Staphylococcus</i> species	13
	2.4.1 Coagulase-Negative Staphylococci Infections	14
	2.4.2 Coagulase-Positive Staphylococci Infections in Dogs and Cats	14
	2.4.3 Coagulase-Positive Staphylococci Infections in other Animals	18
	2.4.4 Coagulase-Positive Staphylococci Infections in Man in Malaysia	20
	2.5 Epidemiology of <i>Staphylococcus</i> Species	21
	2.5.1 Hospital and Community Strains	22
	2.5.2 Nosocomial Infections	23
	2.5.3 Nosocomial Infections in Animals	25
	2.6 Control of Coagulase-Positive Staphylococci	26
	2.6.1 Epidemiological Control	27
	2.6.2 Immunologic Control	28
	2.7 Isolation and Identification of Coagulase-Positive Staphylococci	28
	2.8 Characterisation of Coagulase-Positive Staphylococci	29
	2.8.1 Page Typing	30
	2.8.2 Molecular Analysis	31
	2.8.3 PCR-Based Methods	32
	2.8.4 Random Amplification of Polymorphic DNA (RAPD)	33
	2.8.5 Enterobacterial Repetitive Intergenic Consensus Sequences (ERIC)	35
	2.8.6 Coagulase Gene Polymorphism	36



2.8.7 Protein Profiles	38
2.8.8 Protein A Assay by IgG Binding	39
2.9 Antibiotic Resistance in staphylococci	39
2.10 Genetic Nature of Antimicrobial Resistance	42
2.10.1 Plasmids	43
2.10.2 Plasmid Analysis of Coagulase-Positive Staphylococci	45
2.11 Cluster Analysis	46
3 ISOLATION AND IDENTIFICATION OF COAGULASE-POSITIVE STAPHYLOCOCCI IN DOGS AND CATS	47
3.1 Introduction	47
3.2 Materials and Methods	49
3.2.1 Culture Media	49
3.2.2 Source of Samples	50
3.2.3 Sites of Samples	50
3.2.4 Isolation of Coagulase-Positive Staphylococci	51
3.2.5 Identification of Coagulase-Positive Staphylococci	54
3.2.6 API STAPH	57
3.2.7 The Positive and Negative Control	57
3.2.8 Statistical Analysis	57
3.3 Results	59
3.4 Discussion	68
4 ANTIBIOTIC RESISTANCE AND PLASMID PROFILES	74
4.1 Introduction	74
4.2 Material and Methods	76
4.2.1 Bacterial Isolates Media and Propagation	76
4.2.2 Antimicrobial Susceptibility Tests	77
4.2.3 Preparation of the Inoculum	77
4.2.4 Sensitivity to Antimicrobial Agents	78
4.2.5 The Antimicrobial Agents	78
4.2.6 Multiple Antibiotic Resistance Indexing of Isolates	78
4.2.7 Isolation of Plasmids DNA	79
4.2.8 Visualisation of Plasmids DNA	80
4.2.9 The Molecular Size Marker	81
4.2.10 The Positive and Negative Control	81
4.2.11 Statistical Analysis	81
4.3 Results	82
4.3.1 Antibiotic Sensitivity of <i>S. aureus</i> isolates	82
4.3.2 Antibiotic Sensitivity of <i>S. intermedius</i> isolates	86
4.3.3 Plasmid Analysis of <i>S. aureus</i>	90
4.3.4 Plasmid Analysis of <i>S. intermedius</i>	92
4.4 Discussion	93

5	CHARACTERIZATION OF COAGULASE-POSITIVE STAPHYLOCOCCI BY THEIR PROTEIN PROFILES, AND CONCENTRATION OF PROTEIN A OF WHOLE CELL PROTEIN	102
5.1	Introduction	102
5.2	Material and Methods	103
5.2.1	Preparation of the Proteins	103
5.2.2	SDS Polyacrylamide Gel Electrophoresis	105
5.2.3	Electrophoresis	106
5.2.4	Enzyme Link Immunosorbent Assay (ELISA)	106
5.2.5	Quantification of Protein A	108
5.2.6	Statistical Analysis	108
5.2.7	The Positive and Negative Control	109
5.3	Results	110
5.3.1	Protein Profiles	110
5.3.2	Protein A Assessment	117
5.3.3	Cluster Analysis	119
5.3.4	Cluster Analysis of Protein Profiles	119
5.4	Discussion	122
 6	 CHARACTERIZATION OF COAGULASE-POSITIVE STAPHYLOCOCCI USING POLYMERASE CHAIN REACTION-BASED METHODS	 127
6.1	Introduction	127
6.2	Materials and Methods	130
6.2.1	Isolation of DNA	130
6.2.2	Standardisation of PCR	131
6.2.3	Determination of DNA concentration and Purity	132
6.2.4	Primers for RAPD-PCR	132
6.2.5	RAPD-PCR Conditions	133
6.2.6	Primers for ERIC-PCR	134
6.2.7	ERIC-PCR Conditions	134
6.2.8	Primers for Coagulase gene PCR	135
6.2.9	Coagulase gene PCR Conditions	135
6.2.10	Gel Electrophoresis for Amplified Products of RAPD, ERIC, and Coagulase gene-PCR	136
6.2.11	Visualisation and Photographing of DNA Bands	136
6.2.12	Restriction Fragment Length Polymorphism (RFLP)	136
6.2.13	Statistical Analysis of PCR Methods	137
6.3	Results	138
6.3.1	RAPD-PCR Analysis	138
6.3.2	ERIC-PCR Analysis	144
6.3.3	Coagulase Gene PCR Analysis	147
6.3.4	Restriction Fragment Length Polymorphism of Coagulase gene	151
6.3.5	Cluster Analysis	155

6.3.6 Cluster Analysis of RAPD-PCR	155
6.3.7 Cluster Analysis of ERIC-PCR	158
6.3.8 Cluster Analysis of RFLP	161
6.4 Discussion	168
7 GENERAL DISCUSSION AND CONCLUSION	174
BIBLIOGRAPHY	182
APPENDIX	202
BIODATA OF THE AUTHOR	225

LIST OF TABLES

Table	Page
2.1 <i>Staphylococcus</i> species groups.	7
2.2 Extracellular virulence factors produced by <i>S. aureus</i> and <i>S. intermedius</i>	9
2.3 The main types, function and some examples of plasmid	44
3.1 Biochemical tests for the isolation and identification of <i>S. aureus</i> , <i>S. intermedius</i> and <i>S. hyicus</i> using conventional methods	59
3.2 Frequency of staphylococci isolates from dogs and cats	60
3.3 Frequency of coagulase-positive staphylococci isolates from dogs and cats	60
3.4 Frequency of <i>S. aureus</i> isolates from dogs and cats	61
3.5 Frequency of <i>S. intermedius</i> isolates from dogs and cats	62
3.6 Distribution of staphylococci isolated from three anatomical sites of hospitalised dogs	63
3.7 Distribution of staphylococci isolated from three anatomical sites of outpatient dogs	64
3.8 Distribution of staphylococci isolated from three anatomical sites of hospitalised cats	65
3.9 Distribution of staphylococci isolated from three anatomical sites of outpatient cats	67
4.1 Antibiotic resistance of <i>S. aureus</i> isolates with and without plasmids DNA	83
4.2 Multiple antibiotic resistance and occurrence of plasmids in <i>S. aureus</i> isolates, in four groups of animals	83
4.3 Antibiotic resistance of <i>S. aureus</i> isolates from the four groups of animals	85

4.4	Antibiotic resistance of <i>S. intermedius</i> isolates with and without plasmids DNA	86
4.5	Multiple antibiotic resistance and occurrence of plasmids in <i>S. intermedius</i> isolates on four groups of animals	87
4.6	Antibiotic resistance of <i>S. intermedius</i> isolates from the four groups of animals	90
4.7	Plasmid DNA occurrence in <i>S. aureus</i> isolates in the four groups of animals	91
4.8	Plasmids DNA occurrence in <i>S. intermedius</i> isolates in the four groups of animals	92
5.1	Protein profiles and protein A concentration of <i>S. aureus</i> isolates.	115
5.2	Protein profiles and protein A concentration of <i>S. intermedius</i> isolates.	116
6.1	Distribution of <i>S. aureus</i> isolates according to the RAPD PCR, ERIC PCR, Coagulase gene PCR and RFLP fingerprinting profiles	164
6.2	Distribution of <i>S. intermedius</i> isolates according to the RAPD PCR, ERIC PCR, Coagulase gene PCR and RFLP fingerprinting profiles	165

LIST OF FIGURES

Figure	Page
3.1 The steps used for the isolation of coagulase-positive staphylococci from dogs and cats.	53
3.2 The steps used for the identification of coagulase-positive staphylococci from dogs and cats.	58
4.1 Agarose gel (0.8%) electrophoresis of plasmid DNA from <i>S. aureus</i> isolates 1-13 in Appendix B3. Lane: M, <i>E. coli</i> strain V517 standard marker plasmid of known molecular weight.	91
5.1 Standard curve protein by Lowry Method	104
5.2 Exponential regression analysis of results of ELISA obtained with known concentration of protein A	108
5.3 Protein profiles of <i>S. aureus</i> obtained with SDS-PAGE. Lane: 1, protein marker (kDa). Lane: 2 references strain. Lane: 3,1; 4,2; 5,3 6,4; 7,5; 8,6; 9,7; 10,8; 11,9; 12,10; 13,11; 14,12 correspond to isolate numbers given in Table 5.1.	111
5.4 Protein profiles of <i>S. aureus</i> obtained with SDS-PAGE. Lane: 1, protein marker (kDa). Lane: 2,13; 3,14; 4,15: 5,16; 6,17; 7,18; 8,19; 9,20; 10,21; 11,22; 12,23; 13,24 correspond to isolate numbers given in Table 5.1.	111
5.5 Protein profiles of <i>S. intermedius</i> obtained with SDS-PAGE. Lane: M, protein marker (kDa). Lane: 1 references strain Lane: 2,1; 3,2; 4,3; 5,4; 6,5; 7,6; 8,7; 9,8; 10,9; 11,10; 12,11; 13,12 correspond to isolate numbers given in Table 5.2.	113
5.6 Protein profiles of <i>S. intermedius</i> obtained with SDS-PAGE. Lane: 1, protein marker (kDa). Lane: 2,13; 3,14; 4,15: 5,16; 6,17; 7,18; 8,19; 9,20 10,21; 11,22; 12,23; 13,24 correspond to isolate numbers given in Table 5.2.	113
5.7 Protein profiles of <i>S. intermedius</i> obtained with SDS-PAGE. Lane: 1, protein marker (kDa). Lane: 2,25; 3,26; 4,27: 5,28; 6,29; 7,30; 8,31; 9,32 10,33; 11,34; 12,35; 13,36 correspond to isolate numbers given in Table 5.2.	114

5.8	Protein profiles of <i>S. intermedius</i> obtained with SDS-PAGE. Lane: 1, protein marker (kDa). Lane: 2,37; 3,38; 4,39; 5,40; 6,41; 7,42; 8,43; 9,44 10,45; 11,46; 12,47; 13,48 correspond to isolate numbers given in Table 5.2.	114
5.9	The protein A concentration of <i>S. aureus</i> and <i>S. intermedius</i>	118
5.10	The dendrogram generated from matrix data obtained from SDS Page of whole cell protein. The patristic distance showed the genetic dissimilarity among the <i>S. aureus</i> isolates. R, references isolate. Lane numbers correspond to isolate numbers given in Table 5.1.	120
5.11	The dendrogram generated from matrix data obtained from SDS Page of whole cell protein. The patristic distance showed the genetic dissimilarity among the <i>S. intermedius</i> isolates. R, references isolate. Lane numbers correspond to isolate numbers given in Table 5.2.	121
6.1	RAPD profiles of <i>S. aureus</i> obtained with primer OPA-01 electrophoresed on 1% agarose gel. Lane numbers correspond to isolate numbers given in Table 6.1. Lane M, 1kb molecular weight size marker in base pair.	138
6.2	RAPD profiles of <i>S. aureus</i> obtained with primer OPA-07 electrophoresed on 1% agarose gel. Lane numbers correspond to isolate numbers given in Table 6.1. Lane M, 1kb molecular weight size marker in base pair.	139
6.3	RAPD profiles of <i>S. aureus</i> obtained with primer OPA-08 electrophoresed on 1% agarose gel. Lane numbers correspond to isolate numbers given in Table 6.1. Lane M, 1kb molecular weight size marker in base pair.	140
6.4	RAPD profiles of <i>S. intermedius</i> obtained with primer OPA-01 electrophoresed on 1% agarose gel. Lane numbers correspond to isolate numbers given in Table 6.2. Lane M, 1kb molecular weight size marker in base pair.	141
6.5	RAPD profiles of <i>S. intermedius</i> obtained with primer OPA-07 electrophoresed on 1% agarose gel. Lane numbers correspond to isolate numbers given in Table 6.2. Lane M, 1kb molecular weight size marker in base pair.	142

6.6	RAPD profiles of <i>S. intermedius</i> obtained with primer OPA-08 electrophoresed on 1% agarose gel. Lane numbers correspond to isolate numbers given in Table 6.2. Lane M, 1kb molecular weight size marker in base pair.	143
6.7	ERIC profiles of <i>S. aureus</i> isolates using ERIC1R and ERIC2 primers electrophoresed on 1% agarose gel. Lanes: 2,2; 3,3; 4,4; 5,5; 6,7; 7,8; 8,9; 9,10; 10,11; 11,13; 12,14; 13,15; 14,20; 15,21; 16,22; 17,23; 18,24, correspond to isolate numbers given in Table 6.1. Lane: 1, 1kb molecular weight size marker in base pair.	144
6.8	ERIC profiles of <i>S. aureus</i> isolates using ERIC1R and ERIC2 primers electrophoresed on 1% agarose gel. Lanes: 1,25; 2,27; 3,29; 4,31; 5,33; 6,35; 7,36, correspond to isolate numbers given in Table 6.1. Lane: 8, 1kb molecular weight size marker in base pair.	145
6.9	ERIC profiles of <i>S. intermedius</i> isolates using ERIC1R and ERIC2 primers electrophoresed on 1% agarose gel. Lane: 1,1; 2,2; 3,4; 4,5; 5,6; 6,11; 7,14; 8,15; 9,17; 10,19; 11,22; 12,23; 13,25; 14,27; 15,29; 16,30; 17,32 correspond to isolate numbers given in Table 6.2. Lane: 18, negative control. Lane: 19, positive control. Lane: 20, 1kb molecular weight size marker in base pair.	146
6.10	ERIC profiles of <i>S. intermedius</i> isolates using ERIC1R and ERIC2 primers electrophoresed on 1% agarose gel. Lanes: 1,33; 2,34; 3,35; 4,36; 5,37; 6,39; 7,41; 8,44; 9,47; 10,49; 11,51; 12,55; 13,56; 14,59; 15,62 correspond to isolate numbers given in Table 6.2. Lane: 16, positive control. Lane: 17, negative control. Lane: 18, 1kb molecular weight size marker in base pair.	146
6.11	ERIC profiles of <i>S. intermedius</i> isolates using ERIC1R and ERIC2 primers electrophoresed on 1% agarose gel. Lanes: 1, 64; 2, 67. 3,69; 4,72; 5,75; 6,77; 7,78; 8,80; 9,81; 10,82; 11,83; 12,85; 13,86; 14,89; 15,90 correspond to isolate numbers given in Table 6.2. Lane: 16, positive control. Lane: 17, negative control. Lane 18, 1kb molecular weight size marker in base pair.	147

- 6.12 Coagulase gene profiles of *S. aureus* isolates using COAG2 and COAG3 primers electrophoresed on 1% agarose gel. Lanes: 2,2; 3,3; 4,4; 5,5; 6,7; 7,8; 8,9; 9,10; 10,11; 11,13; 12,14; 13,15; 14,20; 15,21; 16,22; 17,23; 18,24 correspond to isolate numbers given in Table 6.1. Lane 19 positive control. Lane: 20, negatives control. Lane: 1kb molecular weight size marker in base pair. 148
- 6.13 Coagulase gene profiles of *S. aureus* isolates using COAG2 and COAG3 primers electrophoresed on 1% agarose gel. Lanes: 2,25; 3,27; 4,29; 5,31; 6,33; 7,35; 8,36, correspond to isolate numbers given in Table 6.1. Lane: 1, 1kb molecular weight size marker in base pair. 148
- 6.14 Coagulase gene profiles of *S. intermedius* isolates using COAG2 and COAG3 primers electrophoresed on 1% agarose gel. Lane: 1,1; 2,2; 3,4; 4,5; 5,6; 6,11; 7,14; 8,15; 9,17; 10,19; 11,22; 12,23; 13,25; 14,27; 15,29; 16,30; 17,32 correspond to isolate numbers given in Table 6.2. Lane: 18, negative control. Lane 20, 1kb molecular weight size marker in base pair. 149
- 6.15 Coagulase gene profiles of *S. intermedius* isolates using COAG2 and COAG3 primers electrophoresed on 1% agarose gel. Lanes: 1,33; 2,34; 3,35; 4,36; 5,37; 6,39; 7,41; 8,44; 9,47; 10,49; 11,51; 12,55; 13,56; 14,59; 15,62 correspond to isolate numbers given in Table 6.2. Lane: 16, positive control. Lane: 17, negative control. Lane: 18, 1kb molecular weight size marker in base pair. 150
- 6.16 Coagulase gene profiles of *S. intermedius* isolates using COAG2 and COAG3 primers electrophoresed on 1% agarose gel. Lanes: 1,64; 2,67; 3,69; 4,72; 5,75; 6,77; 7,78; 8,80; 9,81; 10,82; 11,83; 12,85; 13,86; 14,89; 15,90 correspond to isolate numbers given in Table 6.2. Lane: 16, positive control. Lane: 17, negative control. Lane 18, 1kb molecular weight size marker in base pair. 150
- 6.17 RFLPs generated by restriction endonuclease analysis of coagulase gene of *S. aureus* isolates using *AluI* restriction enzyme, electrophoresed on 1% agarose gel. Lanes: 2,2; 3,3; 4,4; 5,5; 6,7; 7,8; 8,9; 9,10; 10,11; 11,13; 12,14; 13,15; 14,20; 15,21; 16,22; 17,23; 18,24 correspond to isolate numbers given in Table 6.1. Lane 19 positive control. Lane: 20, negative control. Lane: M, 1kb molecular weight size marker in base pair. 151

6.18	RFLPs generated by restriction endonuclease analysis of coagulase gene of <i>S. aureus</i> isolates using <i>AluI</i> restriction enzyme, electrophoresed on 1% agarose gel. Lanes: 2,25; 3,27; 4,29; 5,31; 6,33; 7,35; 8,36 correspond to isolate numbers given in Table 6.1. Lane: 1, 1kb molecular weight size marker in base pair.	152
6.19	RFLPs generated by restriction endonuclease analysis of coagulase gene of <i>S. intermedius</i> isolates using <i>AluI</i> restriction enzyme, electrophoresed on 1% agarose gel. Lane: 1,1; 2,2; 3,4; 4,5; 5,6; 6,11; 7,14; 8,15; 9,17; 10,19; 11,22; 12,23; 13,25; 14,27; 15,29; 16,30; 17,32 correspond to isolate numbers given in Table 6.2. Lane: 18, negative control. Lane: 19, 1kb molecular weight size marker in base pair.	153
6.20	RFLPs generated by restriction endonuclease analysis of coagulase gene of <i>S. intermedius</i> isolates using <i>AluI</i> restriction enzyme, electrophoresed on 1% agarose gel. Lanes: 1,33; 2,34; 3,35; 4,36; 5,37; 6,39; 7,41; 8,44; 9,47; 10,49; 11,51; 12,55; 13,56; 14,59; 15,62 correspond to isolate numbers given in Table 6.2. Lane: 16, negative control. Lane 17, 1kb molecular weight size marker in base pair.	153
6.21	RFLPs generated by restriction endonuclease analysis of coagulase gene of <i>S. intermedius</i> isolates using <i>AluI</i> restriction enzyme, electrophoresed on 1% agarose gel. Lanes: 1,64; 2,67; 3,69; 4,72; 5,75; 6,77; 7,78; 8,80; 9,81; 10,82; 11,83; 12,85; 13,86; 14,89; 15,90 correspond to isolate numbers given in Table 6.2. Lane 16, negatives control. Lane M, 1kb molecular weight size marker in base pair.	154
6.22	The dendrogram generated from matrix data obtained from RAPD-PCR. The patristic distance showed the genetic dissimilarity among the <i>S. aureus</i> isolates. This is the combined result of the three primers: OPA-01, OPA-07 and OPA-08. Lane numbers correspond to isolate numbers given in Table 6.1.	156
6.23	The dendrogram generated from matrix data obtained from RAPD PCR. The patristic distance showed the genetic dissimilarity among the <i>S. intermedius</i> isolates. This is the combined result of the three primers: OPA-01, OPA-07 and OPA-08. Lane numbers correspond to isolate numbers given in Table 6.2.	157

6.24	The dendrogram generated from matrix data obtained from ERIC-PCR. The patristic distance showed the genetic dissimilarity among the <i>S. aureus</i> isolates. Lane numbers correspond to isolate numbers given in Table 6.1.	159
6.25	The dendrogram generated from matrix data obtained from ERIC-PCR. The patristic distance showed the genetic dissimilarity among the <i>S. intermedius</i> isolates. Lane numbers correspond to isolate numbers given in Table 6.2.	160
6.26	The dendrogram generated from matrix data obtained from RFLP. The patristic distance showed the genetic dissimilarity among the <i>S. aureus</i> isolates. Lane numbers correspond to isolate numbers given in Table 6.1.	162
6.27	The dendrogram generated from matrix data obtained from RFLP. The patristic distance showed the genetic dissimilarity among the <i>S. intermedius</i> isolates. Lane numbers correspond to isolate numbers given in Table 6.2.	163

LIST OF ABBREVIATIONS

Ab	Antibody
AP-PCR	Arbitrary primed polymerase chain reaction
Bp	Base pair
BSA	Bovine serum albumin
°C	Degree Celcius
cm	Centimetre
CNS	Central nervous system
CO ₂	Carbon dioxide
D.W	Distilled water
D.D	De-ionised distilled
DNA	Deoxyribonucleic acid
dNTP	Deoxy-nucleotide triphosphate
Dr.	Doctor
e.g.	For example
EDTA	Ethylene diamine Tetra-acetate
ELISA	Enzyme Link Immunosorbent Assay
ERIC	Enterobacterial repetitive intergenic consensus sequences
g	Gram
G+C	Guanine + Cytisine
H ₂ O ₂	Hydrogen Peroxide
h	Hour
hrs	Hours
HIS	Hyperimmune serum
i.e.	That is
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Kbp	Kilobase pairs
KDa	Kilodalton
M	Molar
Mab	Monoclonal antibody
Mda	Megadalton
M.W.	Molecular weight
mg	Milligram
Min	Minute
Mins	Minutes
ml	Millilitre
mM	Millimole
nm	Nanometer
O.D.	Optical Density
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffer saline